

DEFICIENCY OF LYMPHOCYTE FUNCTION-ASSOCIATED
ANTIGEN 3 (LFA-3) IN PAROXYSMAL NOCTURNAL
HEMOGLOBINURIA

Functional Correlates and Evidence for a Phosphatidylinositol
Membrane Anchor

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Many cell surface glycoproteins are anchored to the membrane via a hydrophobic transmembrane polypeptide segment and a hydrophilic cytoplasmic tail. In contrast, recent studies (1, 2) have shown that some cell surface proteins lack a transmembrane polypeptide domain and are anchored to the cell surface via an unusual glycolipid moiety containing phosphatidylinositol. These proteins can be removed from the cell surface by phosphatidylinositol-specific phospholipase C (PIPLC)¹ (3–8). The significance of this unusual anchorage to the membrane is not yet clear. However, some of these proteins, like Thy-1 and Ly-6/T cell activation protein (TAP) in the mouse, were shown to be involved in T cell activation and signal transduction (9–12). We have examined immunologically important cell surface glycoproteins found on human cells for attachment to the membrane by the phosphatidylinositol glycolipid moiety. For this purpose we have analyzed cells from paroxysmal nocturnal hemoglobinuria (PNH) patients for expression of surface antigens, since recent studies have shown that the molecules deficient in PNH, i.e., acetylcholinesterase (13, 14), decay-accelerating factor (DAF) (15–17), and alkaline phosphatase (18), are anchored to the membrane via a phosphatidylinositol moiety (19–22).

PNH is an acquired bone marrow clonal disorder (23, 24). Patients with PNH have abnormal erythrocytes (E) that are more sensitive to complement-mediated lysis than normal erythrocytes. Three different categories of E have been defined in PNH based on their sensitivity to complement-dependent lysis (25–27). Type I E show normal sensitivity, whereas type II and type III E are 3–5 and 15–25 times more sensitive to complement-mediated lysis, respectively, than type I E.

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¹ *Abbreviations used in this paper:* ACD, anticoagulant citrate dextrose; DAF, decay-accelerating factor; HRF, homologous restriction factor; LFA, lymphocyte function-associated antigen; PIPLC, phosphatidylinositol-specific phospholipase C; PNH, paroxysmal nocturnal hemoglobinuria.

Type II E have been shown to be deficient in the complement regulatory protein called DAF (15–17). Cell surface DAF inhibits the assembly of C3 convertases and thereby regulates the complement cascade (28, 29). In type III E, in addition to DAF, another factor that regulates the assembly of terminal complement components known as homologous restriction factor (HRF) is deficient (30, 31). In PNH patients, neutrophils, monocytes, lymphocytes, and platelets, as well as E, may be deficient in DAF (32, 33), suggesting the defect can arise in a pluripotent stem cell.

Here we show that lymphocyte function-associated antigen 3 (LFA-3) is deficient in PNH cells and can be released from normal cells by PIPLC treatment. LFA-3 is a cell surface glycoprotein of 45–70 kD widely distributed both on hematopoietic and nonhematopoietic cells (34–36). Recent studies have shown that LFA-3 is a ligand for the T cell glycoprotein CD2 (also known as T11, LFA-2, and the E-rosette receptor). Interaction between CD2 and LFA-3 mediates CTL adhesion to target cells (37, 38), thymocyte adhesion to thymic epithelial cells (39), and rosetting of activated T lymphocytes with autologous E (40, 41). We find that LFA-3-deficient PNH E are deficient in adhesion to T cells in the rosetting assay and in binding of ^{125}I -CD2. These defects are corrected by incorporating purified LFA-3 molecules into PNH E.

Materials and Methods

Reagents. FCS, RPMI 1640, HBSS, and gentamycin were from Gibco Laboratories, Grand Island, NY. FCS was heat inactivated at 56°C for 30 min before use. FITC-conjugated goat anti-mouse IgG was purchased from Zymed Laboratories, San Francisco, CA. OVA (grade V) and aprotinin were purchased from Sigma Chemical Co., St. Louis, MO. Anticoagulant citrate dextrose (ACD) was from Fenwal Laboratories, Deerfield, IL. mAbs used were: TS2/9 specific for LFA-3 (34), 1A10 specific for DAF (33), L180/1 specific for sheep T11TS (42), W6/32 (HLA-AB) (43), TS1/22 (LFA-1) (34), TS2/18 (CD2) (34), OKMI (Mac-1) (Ortho Pharmaceutical, Raritan, NJ), Leu-2a (CD8), and Leu-3a (CD4) (Becton Dickinson & Co., Mountain View, CA). P3 X63, a myeloma IgG, was used as a nonbinding control. All washings, incubations, and dilutions were done with 10% FCS/RPMI. PIPLC was purified from *Staphylococcus aureus* bacterial culture supernatant as described (44).

Patients and Controls. Blood samples were collected from PNH patients and from normal individuals either in ACD or in Heparin (10 U/ml) as anticoagulants. PNH patients DE and PK had type III E. Patients SB, MW, and CP had type II E. Patient JB had both type II and type III E. As a control, blood was drawn from healthy donors at the same time and processed similarly.

Cells. Leukocyte-rich plasma was separated from E by 6% dextran (Pharmacia Fine Chemicals, Piscataway, NJ) sedimentation. The separated E were stored as such at 4°C up to 1 mo if the blood was collected in ACD or washed five times with 10% FCS/RPMI/gentamycin and resuspended in the same medium before storage if the blood was collected in heparin. E were washed three to five times with 10% FCS/RPMI/gentamycin before use. Jurkat cells, a human T lymphoma cell line, were grown in 10% FCS/RPMI/gentamycin and washed thrice with 10% FCS/RPMI/gentamycin before use in the rosetting assay.

Immunofluorescence Flow Cytometry. E were washed three to five times and resuspended at a concentration of 5×10^7 cells/ml. An aliquot of 50 μl of cells (5×10^6 cells) were incubated with an equal volume of specific antibody for 30 min at 4°C. Specific antibodies in hybridoma culture supernatants or in diluted ascites were used at saturating concentrations. The cells were washed thrice and further incubated with FITC-conjugated goat anti-mouse IgG for 30 min at 4°C. Then the cells were washed thrice, resuspended, and

analyzed in an Epics V flow cytometer (Coulter Electronics Inc., Hialeah, FL). A minimum of 10,000 cells were analyzed per experiment. As a nonspecific control, X63, a nonbinding myeloma IgG, was used.

¹²⁵I-CD2 Binding Assays. ¹²⁵I-labeled purified CD2 antigen was prepared as previously described (38). The ¹²⁵I-CD2 binding assays were carried out as follows: washed E (5×10^6 cells) were incubated with either 50 μ l of antibody or 50 μ l of 10% FCS/RPMI for 45 min at 4°C in a microtiter plate pretreated with 10% FCS/RPMI. Then 50 μ l of varying concentrations of ¹²⁵I-CD2 diluted in 3% BSA/10% FCS/RPMI was added and the incubation was continued for another 2 h at 4°C with continuous shaking. The cells were washed thrice with 10% FCS/RPMI, resuspended, and counted in a gamma counter. Under the experimental conditions used no lysis of E was observed during the binding studies.

Rosetting Assay. Rosetting of Jurkat cells with human E was carried out as described (41). Briefly, 100 μ l of 10^8 Jurkat cells/ml were mixed with 100 μ l of 10^8 E/ml at 37°C for 5 min and incubated for 2 h at 4°C. The cells were gently resuspended and Jurkat cells rosetting with a minimum of three E were scored as rosettes. Because of the day-to-day variations in the percent of Jurkat cells rosetting with control human E, rosetting of PNH E was always compared with the rosetting of normal control E counted in parallel.

Incorporation of Purified LFA-3 into E. LFA-3 was purified from human E to homogeneity using TS2/9-Sepharose affinity chromatography as described (40). The LFA-3 eluate (50 μ g/ml in nine parts 50 mM glycine/HCl buffer, pH 2.5, 0.1% Triton X-100, and one part of 1 M Tris/HCl, pH 8.6) was diluted 100 times with HBSS, added to packed E to give a final concentration of 10^8 cells/ml, and incubated for 4 h at 37°C during gentle end-over-end rotation. The tube was filled except for a small air bubble. As a control, an aliquot of PNH or normal E was incubated in parallel with an identical buffer lacking LFA-3. After incubation the E were washed thrice with 10% FCS/RPMI. Under these conditions no cell lysis was observed.

PIPLC Treatment of Cells. Washed E or JY cells, a B lymphoblastoid cell line, were incubated with HBSS/2 mM Hepes, pH 7.4, or PBS, pH 7.4, containing 1 mg/ml OVA and 10 μ g/ml of PIPLC at 37°C for 1 h. Then the cells were washed and analyzed for surface antigens by immunofluorescence flow cytometry or immunoprecipitation. As a control, the cells were incubated under identical conditions but without PIPLC.

Immunoprecipitation and SDS-PAGE. JY cells were surface iodinated with ¹²⁵I using 1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycoluril as described (45). Aliquots of the labeled cells were incubated with or without PIPLC as described above for 1 h at 37°C and centrifuged at 1,000 g for 7 min. The supernatant was further centrifuged at 100,000 g for 1 h and saved for immunoprecipitation. The 1,000 g cell pellet was washed with HBSS and lysed in 1% Triton X-100 in 10 mM Tris/HCl/145 mM NaCl/0.02% NaN₃, pH 8.0, containing 1% bovine hemoglobin, 1 mM iodoacetamide, 1 mM PMSF, and 1% aprotinin. The LFA-3 in the supernatants and the lysates was immunoprecipitated with TS2/9-Sepharose and subjected to SDS-PAGE (46) under reducing conditions. The gel was dried and autoradiographed.

Results

Deficient LFA-3 Expression on PNH E. Patient DE was deficient in DAF in E, lymphocytes, granulocytes, and monocytes as previously described (33). mAb staining of these cells in patient DE showed normal expression of LFA-1, CD2, CD4, CD8, and HLA-A, B. However, DE E were almost completely deficient in LFA-3 (Fig. 1A). Deficiency of LFA-3 on E was verified in further PNH patients (Fig. 1B-E). The severity of LFA-3 deficiency varied. For patient DE, nearly 90% of E had little or no LFA-3 and 10% had subnormal levels of LFA-3 (Fig. 1A). Patient SB showed three different populations. ~10% of the E expressed a normal level of LFA-3, 70% of the E expressed a subnormal amount of LFA-3 and 20% showed no LFA-3 expression (Fig. 1B). Patients JB, MW, and CP had

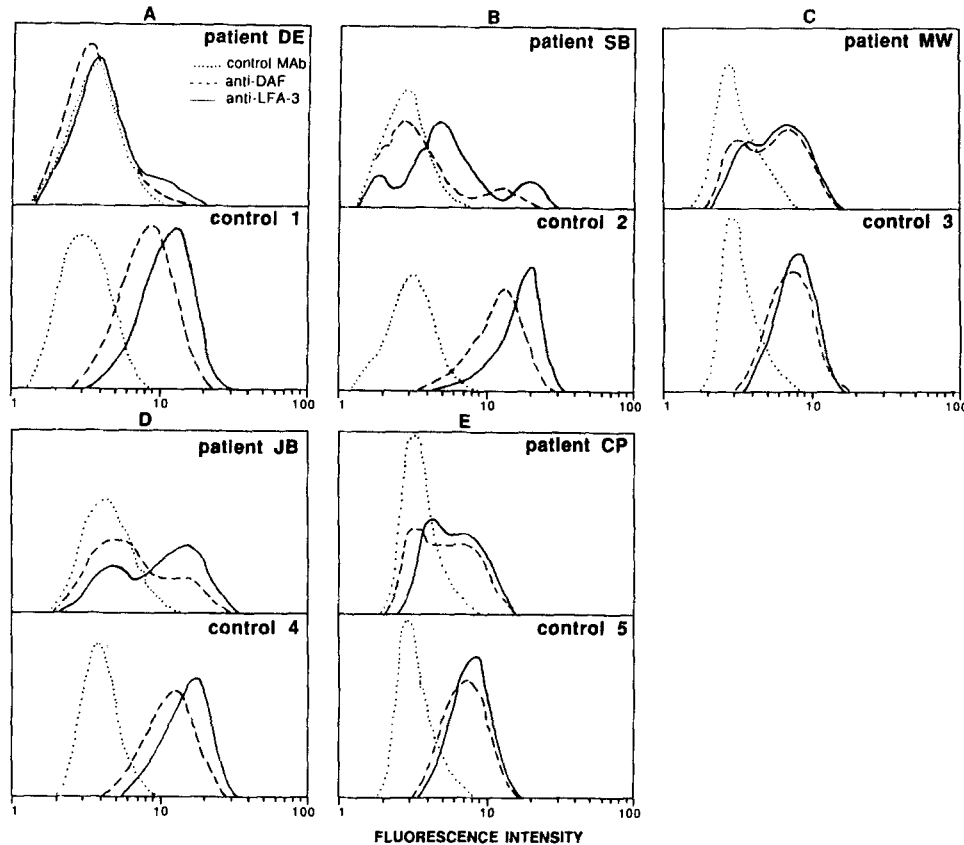


FIGURE 1. Fluorescent staining of LFA-3 and DAF on PNH and healthy control E. E were incubated with LFA-3 mAb (solid lines), anti-DAF mAb (dashed lines), or X63, control IgG (dotted lines), stained with fluorescein-labeled goat anti-mouse IgG, and subjected to immunofluorescent flow cytometry. Matched controls are shown for each patient since fluorescence amplification settings varied for some of the patients.

two distinct populations; one totally deficient in LFA-3, and a second population with subnormal or normal amounts of LFA-3. DAF deficiency paralleled LFA-3 deficiency, but was not always identical to that of LFA-3. LFA-3 deficiency was not as marked as DAF deficiency in patients JB, SB, and CP.

¹²⁵I-CD2 Binding to PNH Erythrocytes. Previous studies (37-41) have shown that the T lymphocyte surface glycoprotein CD2 is a receptor for LFA-3. Detergent-solubilized, purified CD2 binds to cell surface LFA-3 (38, 41). PNH erythrocytes showed reduced binding of ¹²⁵I-CD2 (Fig. 2). Compared with controls, E from patient DE showed 13% ¹²⁵I-CD2 binding, whereas E from patients PK, JB, and SB showed 48, 44, and 33% ¹²⁵I-CD2 binding. Binding of CD2 by PNH E correlated with LFA-3 expression (compare Fig. 1 and Fig. 2).

Rosetting of PNH E with Jurkat Cells. Previously we have shown (40, 41) that the autologous rosetting of human T lymphocytes with E is mediated by the CD2 and LFA-3 receptor-ligand interaction. We examined whether the decreased LFA-3 expression on PNH E correlated with a defect in adherence (rosetting) to T cells (Table I). The T lymphoma cell line Jurkat was used in

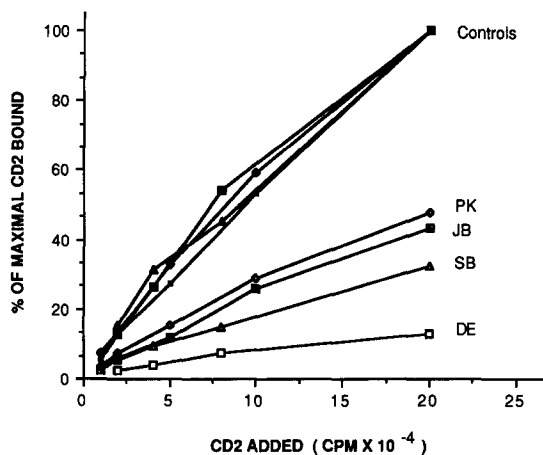


FIGURE 2. ^{125}I -CD2 binding to PNH E and control E. PNH and control E (5×10^6) were incubated with subsaturating concentrations of ^{125}I -CD2 (input of 100,000 cpm) and the binding assay was carried out as described in Materials and Methods. The amount of CD2 bound to normal control E at the highest concentration of added ^{125}I -CD2 was taken as 100%. The specific activity of ^{125}I -CD2 was 3.8×10^8 cpm/nmol.

TABLE I
PNH E are Deficient in T Lymphocyte Rosetting

| Exp. | Patient | Rosetting Jurkat T lymphoma cells | | Patient E (percent of control) |
|------|---------|-----------------------------------|-----------|--------------------------------|
| | | Control E | Patient E | |
| | | % | | |
| 1 | DE (1)* | 52 | 3 | 6 |
| 2 | DE (2)* | 42 | 4 | 11 |
| 3 | SB | 71 | 11 | 20 |
| 4 | PK | 69 | 28 | 38 |
| 5 | JB | 51 | 22 | 43 |
| 6 | MW | 66 | 44 | 67 |
| 7 | CP | 61 | 31 | 51 |

* DE (1) and DE (2) are E obtained from the same patient at two different blood donations with 7-wk intervals.

rosetting assays because it is a higher expressor of CD2 (47), and rosettes well with human E (41). PNH E were deficient in the rosette assay, and the degree of deficiency in rosetting correlated with the extent of LFA-3 deficiency. Rosettes with PNH E also contained fewer E, ranging from 3–5 for DE and SB, compared with 5–20 for control E.

Correction of Functional Defects by Incorporation of Purified LFA-3 into PNH E. When exogenous, purified LFA-3 (0.5 $\mu\text{g}/\text{ml}$) was incubated with PNH or control E at 37°C it was readily incorporated into the E membrane (Fig. 3). The incorporated LFA-3 was stable on the cell surface and was not removable by repeated washing with PBS, HBSS, or 10% FCS/RPMI. Storage of washed E at 4°C for up to 1 wk did not result in loss of incorporated LFA-3 (data not shown). LFA-3 could be incorporated into PNH E (Fig. 3, A–C, bottom panels) in higher amounts than found on untreated control E (Fig. 3D, top panel). Incorporation of exogenous LFA-3 did not alter staining with DAF mAb (Fig. 3). This demonstrates that LFA-3 and DAF are different molecules. These molecules also differed in side-by-side SDS-PAGE; LFA-3 is a diffuse band of 43–68 kD, whereas DAF is a sharp band of 70 kD (data not shown).

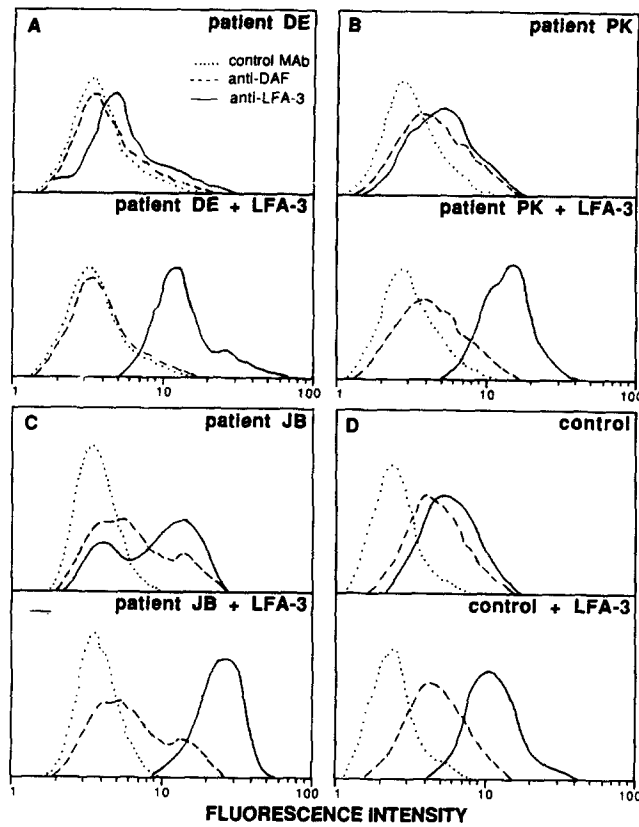


FIGURE 3. Incorporation of exogenous LFA-3 into PNH and control E. E were incubated with purified LFA-3 or with control buffer as described under Materials and Methods. Washed cells were stained with LFA-3 mAb (solid lines), or anti-DAF mAb (dashed lines) or control IgG (dotted lines), and fluorescein goat anti-mouse IgG.

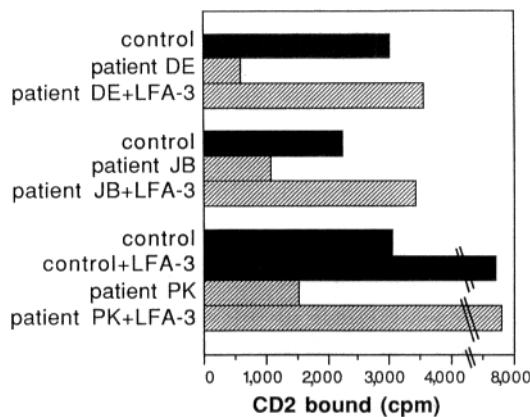


FIGURE 4. Incorporation of LFA-3 into PNH E and healthy control E augments ^{125}I -CD2 binding. LFA-3 was incorporated into E and ^{125}I -CD2 binding was carried out as described under Materials and Methods. 5×10^6 cells and 100,000 cpm of ^{125}I -CD2 were used per assay point. Each point represents the average of duplicates. Binding of ^{125}I -CD2 after pretreatment of cells with LFA-3 mAb was tested in all experimental groups and was $x = 6\%$, range = 0-14% of binding in the absence of pretreatment.

We determined whether functional defects in ability to bind purified CD2 and rosette with T lymphocytes were due to deficiency of LFA-3 or to the deficiency of other affected phosphatidylinositol-linked surface proteins. The reconstituted cell preparations analyzed for LFA-3 incorporation in Fig. 3 were tested for CD2 binding (Fig. 4). Reconstitution of DE, JB, and PK E with exogenous LFA-3 reconstituted ^{125}I -CD2 binding. Addition of exogenous LFA-3 to normal E also resulted in increased ^{125}I -CD2 binding. The amount of ^{125}I -CD2 binding corre-

TABLE II
Incorporation of Exogenous LFA-3 Reconstitutes Rosetting

| Exp. | Patient | Percent rosetting after treatment | |
|------|------------|-----------------------------------|--------|
| | | None | +LFA-3 |
| 1 | Control | 32 | 68 |
| | Patient PK | 12 | 53 |
| 2 | Control | 56 | ND |
| | Patient JB | 26 | 68 |
| 3 | Control | 74 | 81 |
| | Patient MW | 45 | 85 |
| | Patient CP | 28 | 81 |

Patient or control E were incubated with purified LFA-3 and rosetted with Jurkat T lymphoma cells as described under Materials and Methods.

lated with the amount of cell surface LFA-3 (compare Fig. 3 and Fig. 4). The binding of ^{125}I -CD2 was completely inhibited by pretreating the E with anti-LFA-3 antibody, indicating that it was due to specific binding of ^{125}I -CD2 to cell surface LFA-3.

Incorporation of LFA-3 into PNH E reconstituted E rosetting to Jurkat cells (Table II). The percentage of Jurkat cells rosetting with E correlated with the amount of LFA-3/E, and LFA-3 reconstituted in PNH E to higher levels than present on untreated controls resulted in a higher percentage of rosetting than with untreated control E. Incorporation of exogenous LFA-3 into control E also augmented rosetting.

PIPLC Treatment of Cells. LFA-3 deficiency in PNH suggested that this antigen is attached to cell membranes via a glycolipid tail containing phosphatidylinositol. Nearly 35% of LFA-3 was removed from the surface of JY (a B lymphoblastoid cell line) cells by PIPLC treatment (Fig. 5A). The decrease in cell surface LFA-3 after PIPLC treatment was specific because other membrane proteins like HLA-A, B and LFA-1 were not affected, whereas, under the same conditions nearly 62% of the DAF was released from the membrane. PIPLC treatment of ^{125}I -labeled cells confirmed that the released LFA-3 could be recovered from the supernatant by immunoprecipitation (Fig. 6).

PIPLC treatment did not release a significant amount of endogenous LFA-3 from E (Fig. 5B); however, E DAF and acetylcholinesterase, which have been shown to be linked via phosphatidylinositol to the E cell membrane, are also resistant to PIPLC treatment on E (Fig. 5B, references 5, 20, 21, 48). DAF is released from other cell types as reported previously (5) and confirmed in Fig. 5A. Some feature, either of the E surface or a specific modification of the glycolipid moiety in E may render them resistant to PIPLC. However, when purified erythrocyte DAF is incorporated into sheep E it becomes susceptible to PIPLC treatment (21). We tested whether purified erythrocyte LFA-3 incorporated into sheep or human E was sensitive to PIPLC. Nearly 50% of LFA-3 incorporated into sheep E was released by PIPLC, whereas under the same conditions sheep T11TS, the sheep homologue of LFA-3, was unaffected (Fig. 5C). Purified LFA-3 incorporated into human healthy control or PNH E was similarly susceptible to PIPLC treatment (Fig. 5D).

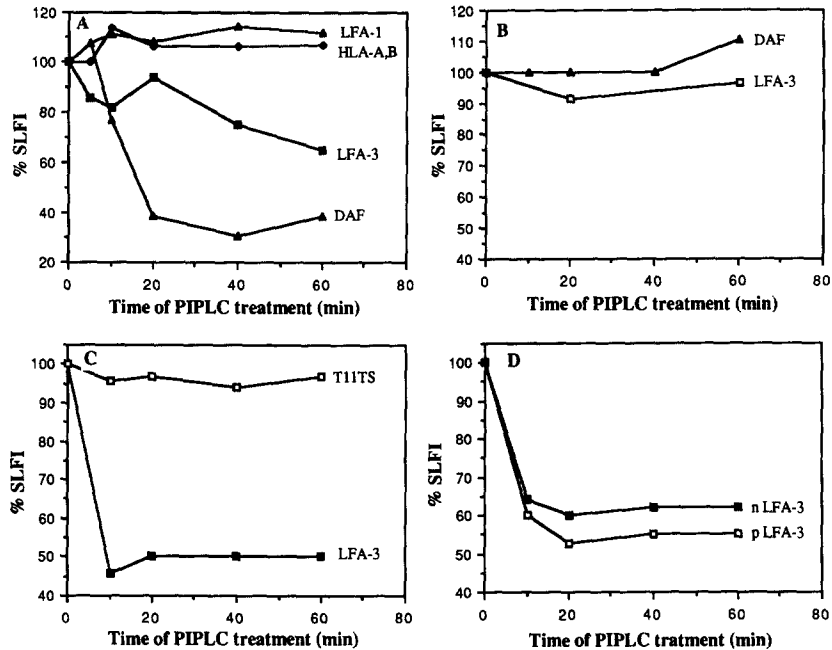


FIGURE 5. PIPLC treatment of JY cells and E. Cells were treated with 10 $\mu\text{g}/\text{ml}$ of PIPLC as described in Materials and Methods at 37°C. The cells were washed and incubated with specific or control mAb, then stained with fluorescein-labeled goat anti-mouse IgG. Labeled cells were analyzed by immunofluorescence flow cytometry. The specific linear fluorescence (SLFI) of cells treated under the same conditions but without PIPLC was taken as 100%. (A) JY cells. (B) Healthy control human E. (C) Sheep E with incorporated, purified LFA-3. (D) Human E with incorporated, purified LFA-3 (open squares, PNH E treated with LFA-3; closed squares, control E treated with LFA-3).

Discussion

We have found that LFA-3 is deficient on E from patients with PNH, an acquired hematopoietic clonal defect that affects the cell surface expression of phosphatidylinositol-anchored membrane proteins. Previous studies (37-41) have shown that LFA-3, a widely distributed cell surface glycoprotein, is a ligand of the T lymphocyte surface glycoprotein CD2. Binding of CD2 to LFA-3 mediates antigen-independent adhesion of T lymphocytes to many types of cells including target cells, thymic epithelial cells, and E. Adhesion via CD2/LFA-3 is regulated by a number of factors including cell surface density of CD2 and LFA-3, and by the cell surface charge (49). CD2⁺ resting T lymphocytes do not rosette with autologous LFA-3⁺ E, but do form rosettes if sialic acid is removed from either cell. Furthermore, activated T lymphocytes and thymocytes, which have more CD2 (50, 51) and two- and fivefold less sialic acid (52) than resting T lymphocytes, respectively, readily rosette with human E. The previous conclusion that CD2 binds to LFA-3 was based on competition between purified CD2 and LFA-3 mAb for binding to cell surface LFA-3, and reciprocally, competition between purified LFA-3 and CD2 mAb for binding to cell surface CD2 (38, 40, 41). Deficiency of LFA-3 on PNH E provided an independent means of testing the interaction of CD2 with LFA-3, which to our knowledge is the first demon-

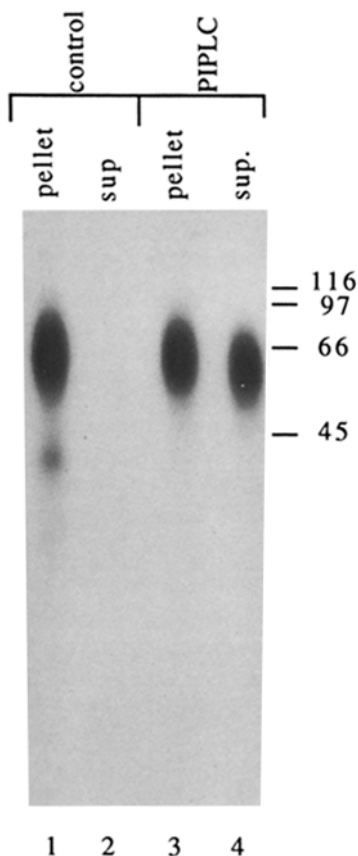


FIGURE 6. SDS PAGE of LFA-3 released from cells by PIPLC. Surface-iodinated JY cells (10^6 cells) were treated with 0.28 M sucrose/25 mM HEPES buffer, pH 7.4, with or without PIPLC (10 μ g/ml) for 1 h at 37°C. LFA-3 was immunoprecipitated from the supernatants and cell lysates and subjected to SDS-12% PAGE under nonreducing conditions. The gel was dried and autoradiographed.

stration of an integral membrane adhesion receptor which binds a distinct integral membrane ligand. E from our most severely affected PNH patient, which were 91% LFA-3 deficient, showed quantitatively similar defects in binding of 125 I-CD2 and rosetting with T lymphoma cells. Overall in six different PNH patients that had varying proportions of affected E populations, there was an excellent correlation between amount of cell surface LFA-3, 125 I-CD2 binding, and rosetting.

Similarly to DAF, which has a phosphatidylinositol membrane anchor (5, 21, 29), we found that purified LFA-3 could be readily incorporated into the membrane bilayers of both PNH and control E. Incorporation of purified LFA-3 into PNH E corrected the defects in 125 I-CD2 binding and rosetting. Incorporation into normal E, resulting in increased cell surface LFA-3, augmented 125 I-CD2 binding and rosetting. The findings that LFA-3-deficient PNH E are correspondingly deficient in binding purified CD2 and in rosetting with T lymphocytes, and that reconstitution with LFA-3 corrects these defects, strongly supports previous conclusions that CD2 binds LFA-3 (37-41) and that this interaction mediates rosetting of T lymphocytes with autologous E (41).

PIPLC specifically released LFA-3 from B lymphoblastoid cells, as determined both by immunofluorescence and by immunoprecipitation. These results con-

firmed that LFA-3 is anchored to the membrane via phosphatidylinositol. PIPLC did not release endogenous LFA-3 from E, but purified E LFA-3 that was incorporated into sheep or human E was released by PIPLC. Whether this is due to removal of an associated molecule or to cleavage of some portion of the glycolipid moiety during purification, or reflects a difference between endogenous and exogenous LFA-3 in the way they are incorporated in the membrane, is not known. Endogenous E DAF, which has been shown chemically to be linked to phosphatidylinositol, is also largely PIPLC resistant (5, 21). Reports conflict on whether exogenous DAF incorporated into E is sensitive to PIPLC (5, 21).

Using an excess of PIPLC, we consistently found a higher proportion of DAF than LFA-3 was released from lymphoblastoid cells (62 vs. 35%). Since the work reported here was completed, two lines of investigation have suggested that on white blood cells LFA-3 is present in two forms, a phosphatidylinositol-linked form and a distinct, putative hydrophobic polypeptide chain-linked form, whereas on E predominantly the phosphatidylinositol-linked form is present. First, on PNH monocytes and granulocytes that appear to be clonally affected as shown by complete absence of DAF, LFA-3 is expressed but at slightly reduced levels (Medof, M. E., P. Selvaraj, M. L. Dustin, D. G. Ayers, E. I. Walter, H. A. Stafford, R. Green, M. L. Tykocinski, and T. A. Springer, manuscript in preparation). Secondly, biosynthesis experiments have demonstrated two forms of LFA-3 that differ in size by 3 kD on many different types of nucleated cells. The lower but not the higher molecular mass form is released from the cell surface by PIPLC (Dustin, M. L., P. Selvaraj, R. J. Mattaliano, and T. A. Springer, submitted for publication).

Molecules deficient in PNH erythrocytes or leukocytes include DAF (15–17), a protein important in assembly of the C8, C9 complex called HRF (30, 31), acetylcholinesterase (13, 14), alkaline phosphatase (18), and now LFA-3. Linkage of four of these proteins to phosphatidylinositol (linkage of HRF has not yet been tested) suggests that the primary defect in PNH is related to the assembly or the linkage of the phosphatidylinositol glycolipid moiety. The clinical symptoms in PNH appear due to absence of complement regulatory proteins DAF (15–17) and HRF (30, 31), resulting in complement-mediated lysis of red blood cells, and increased hematopoiesis. Although some impairment of immune function has been reported (53, 54), it is unclear whether this is related to deficiency of LFA-3. Only one form of LFA-3 is deficient on white blood cells in PNH, as mentioned above, and LFA-3 is expressed on some nonhematopoietic cell types including endothelial and epithelial cells, which are unaffected in PNH.

The phosphatidylinositol glycolipid anchor of LFA-3 may be of physiologic importance. The lateral mobility of two studied phosphatidylinositol-linked proteins, DAF and Thy-1, is higher than for surface proteins with transmembrane polypeptide chains (55, 56). Mutual redistribution of CD2 and LFA-3 to a site of adhesion would be dependent on the ability of the receptor and ligand to diffuse into the site of cell–cell contact. Thus, the predicted high rate of diffusion of LFA-3 may make it more effective in mediating cell adhesion. The phosphatidylinositol anchor may also be important in signaling. Recent studies (57) have shown that the CD2- and LFA-3-dependent interaction of thymocytes with thymic epithelial cells stimulates the latter cell type to secrete IL-1. Furthermore,

LFA-3 mAb mimics this by inducing IL-1 secretion by thymic epithelial cells and monocytes (57). Whether signal transduction by LFA-3 or by other glycolipid-anchored surface proteins (6, 10–12) is mediated by the phosphatidylinositol moiety remains to be determined.

Summary

Lymphocyte function-associated antigen 3 (LFA-3) is a widely distributed cell surface glycoprotein that binds to the T lymphocyte CD2 surface glycoprotein. This interaction mediates CTL–target cell conjugate formation and adhesion of thymocytes to thymic epithelial cells. CD2 is also the E rosette receptor of T lymphocytes and mediates rosetting with autologous E by binding to LFA-3. We describe deficient expression of LFA-3 on E from paroxysmal nocturnal hemoglobinuria (PNH) patients. PNH is an acquired defect affecting phosphatidylinositol-anchored membrane proteins, of which decay-accelerating factor (DAF) is most important in the clinical symptoms of PNH. LFA-3-negative, weakly positive, and positive populations were found among PNH E. There was a good correlation with DAF deficiency. PNH E exhibited decreased binding of ^{125}I -CD2 and rosetting with a human T lymphoma cell line. PNH E readily incorporated purified LFA-3, restoring LFA-3 expression and the CD2 binding and rosetting activity to normal levels. The expression of DAF was not restored after the incorporation of purified LFA-3 into PNH E, showing that LFA-3 and DAF are different molecules. Phosphatidylinositol-specific phospholipase C (PIPLC) treatment of a B lymphoma cell line released 35% of the cell surface LFA-3 and 62% of DAF. LFA-3 on E was resistant to PIPLC. However, when LFA-3 purified from human E was reconstituted in sheep E or human E and subjected to PIPLC treatment, 40–50% of LFA-3 was released from the cell membrane. The results show that LFA-3 is attached to the cell membrane by a phosphatidylinositol glycolipid moiety, and confirm previous findings (37–41) that LFA-3 is a cell adhesion molecule that mediates adhesion by interacting with CD2 antigen.

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